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Research Article

Temporal variation in hotspots and the effects of climate and landscape on wildlife roadkill along the BR-319: conservation challenges in a high-biodiversity area in the Amazon

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Abstract

Roads greatly impact wildlife, with roadkill posing a major threat to many species. In the Amazon, highways cut through biodiversity-rich ecosystems, exposing mammals, birds, reptiles, and amphibians to roadkill risks. Increasing traffic and expanding road networks drive population declines, influenced by environmental traits, traffic, and seasonal conditions. Temporal variation in these conditions can lead to shifting roadkill patterns over time, making it essential to understand how such changes affect wildlife mortality. Our study aims to identify temporal variation on roadkill hotspots and investigate their relationship with climatic (temperature and rainfall) and landscape (forest, agricultural, and wetland cover) factors. We used a roadkill database from the first 177.8 km of BR-319 (Manaus – Porto Velho) spanning 2017 to 2021 to evaluate variations in hotspots over time and investigate how those climate and landscape factors affect roadkills across different buffers (500 m, 1 km, 5 km, and 10 km). We recorded 673 roadkill incidents (107 mammals, 251 reptiles, 152 birds, and 163 amphibians). Hotspot patterns varied across years, with amphibians, reptiles, birds, and mammals showing distinct hotspot patterns. Climate and landscape factors significantly influenced roadkill rates, with higher temperatures correlating with more roadkill, while rainfall generally decreased incidents, and increased forest and water cover helped reduce roadkill across most groups. Our findings suggest that both climatic and landscape factors play crucial roles in shaping roadkill patterns, reinforcing the need to monitor changes over time and highlighting the need for context-specific conservation strategies along highways.

* The opinions expressed in this article are the sole responsibility of the author and do not necessarily reflect his official position at the Brazilian Ministry of Environment and Climate Change.

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In conclusion, mitigating roadkill along BR-319 will require integrated approaches that consider environmental variables, traffic management, and habitat protection to reduce wildlife mortality.

Key words: Amazon highways, road ecology, wildlife mortality, wildlife-vehicle collisions

Introduction

Projections indicate that by 2050, an additional 25 million kilometers of paved roads will be constructed globally compared to 2010, representing more than 60% increase in global road coverage (Dulac 2013). While this expansion may boost economic development, it poses significant environmental risks, particularly in high-biodiversity and conserved regions, since roads directly contribute to habitat fragmentation and indirectly exacerbate activities such as logging and land occupation (Asher et al. 2020; Barrientos et al. 2021). For biodiversity, one of the most immediate effects is the increase in wildlife roadkill, which drives declines in numerous species' populations and ecosystems' functionality (Laurance et al. 2009).

The dynamics of wildlife roadkill are shaped by environmental factors (e.g., forest cover, proximity to water bodies), road characteristics (e.g., number of lanes, speed limits), climatic variables (e.g., temperature, precipitation, seasonality), and species-specific traits (e.g., mobility, activity, and reproductive periods) (González-Suárez et al. 2018). These elements interact to create critical areas, or hotspots, with high concentrations of wildlife roadkill, which have been studied in both open and forested habitats across tropical and temperate zones to inform the placement of mitigation measures (e.g., Ha and Shilling 2018; Marques et al. 2022; Santos et al. 2022; Pinto et al. 2023). However, how these roadkill hotspots shift over time remains poorly understood. Seasonal events, such as migrations, reproductive periods, or fluctuations in water levels, can shift hotspot locations (Brum et al. 2017; Ferreguetti et al. 2020). Additionally, land-use changes, including deforestation or urbanization, may cause hotspots to emerge or disappear over time (Grilo et al. 2011; Medrano-Vizcaíno et al. 2022). The effects of climate and landscape variables with roadkills is dynamic and complex, influencing when and where roadkill events are most likely to occur. Understanding how these factors act is crucial for identifying critical periods of risk.

Climatic factors such as rainfall and temperature, together with land-use changes like agricultural expansion and urbanization, interact to influence the spatial and temporal distribution of wildlife-vehicle collisions (Medinas et al. 2021; Kouris et al. 2024). Understanding these temporal patterns is critical, as certain periods may present elevated risks due to species behavior and environmental conditions. This knowledge is key to implementing time-sensitive mitigation strategies, such as temporary wildlife crossings, seasonal speed limits, or targeted awareness campaigns during peak collision periods (e.g., breeding seasons or flooding) (Gonçalves et al. 2023).

Landscape composition and configuration play a critical role in shaping collision rates, either amplifying or reducing them (Grilo et al. 2011). Fragmented landscapes with dense road networks often experience higher collision rates due to increased animal movement between habitat fragments. For example, carnivores are frequently impacted in agricultural regions, drawn by the abundance

of prey in croplands, but the specific responses tend to be very species-specific (Heim et al. 2019). Amphibians are particularly vulnerable near water bodies, as road crossings become essential during reproductive periods (Taylor et al. 2020). Overall, the presence of dense forests, low vegetation, and agricultural areas affects the susceptibility of various faunal groups, including birds and mammals, to collisions (Medrano-Vizcaíno et al. 2022). The interaction between roads, landscapes, and wildlife-vehicle collisions also varies with the scale of observation, as species responses are shaped by ecological processes that operate at multiple scales (Jackson and Fahrig 2015; González-Suárez et al. 2018). Identifying the optimal scale for analyzing landscape composition and configuration remains a challenge due to this multiscale complexity. To address this, a multiscale approach is needed to determine the specific scale at which each variable has the greatest effect in the wildlife roadkill (Jackson and Fahrig 2015).

Climatic factors also influence collision rates, particularly in tropical regions. High temperatures and precipitation levels often increase the mobility of reptiles and amphibians, heightening their susceptibility to road mortality, especially during reproductive periods when their movement in search of mates or nesting sites intensifies (Carvalho et al. 2017; Medrano-Vizcaíno et al. 2023). In contrast, birds and mammals show an indirect relationship with climatic variables, as their collision rates are typically seasonal, often associated with reproductive periods and a higher presence of young, inexperienced individuals (Husby 2016; Ferreguetti et al. 2020). Rainfall can further influence collision rates by altering food availability, population abundance, and species movement patterns (Machado et al. 2015). While increased collisions during the rainy season have been previously observed (Machado et al. 2015), this pattern is not universal. For instance, research conducted in Carajá, found no significant correlation between rainfall and wildlife roadkill (Gumier-Costa and Sperber 2009).

While general patterns of wildlife-vehicle collisions are well-documented, local and regional factors play a crucial role, highlighting the need for mitigation strategies tailored to specific contexts. This issue is particularly urgent in the Amazon, a region known for its high ecological heterogeneity and exceptional biodiversity (Medrano-Vizcaíno et al. 2023). Most studies on wildlife roadkill have been conducted in temperate zones or tropical and subtropical areas that are either non-forested or heavily fragmented (Jackson and Fahrig 2015; Grilo et al. 2021). In contrast, the Amazon remains relatively understudied in this context, especially regarding the temporal variability of roadkill and its drivers. This creates a significant knowledge gap in understanding how roadkill risk fluctuates across time and space in intact forest landscapes undergoing rapid change.

Although some isolated studies in the Amazon have evaluated wildlife roadkill (Carvalho et al. 2015; Batista et al. 2022; Santos et al. 2022),they often lack a temporal or multiscale landscape perspective. Adeeper understanding of roadkill patterns is necessary to design effective conservation measures for the Amazon (Filius et al. 2020; Medrano-Vizcaíno et al. 2022). Quantifying the impacts of road expansion on Amazonian biodiversity is therefore critical for developing strategies to minimize biodiversity loss and safeguard the ecosystem services provided by the Amazon at both regional and global scales (Fearnside et al. 2020). By incorporating both temporal and spatial components, our study offers new insights into how environmental drivers influence wildlife-vehicle collisions over time, providing essential information for adaptive conservation policy in a rapidly changing region.

In this context, the BR-319 highway, which connects Manaus to Porto Velho, is a strategic road for Brazil's northern region, cutting environmentally sensitive areas, including protected areas and Indigenous lands (Fearnside et al. 2020). Its revitalization, including the paving of over 400 km cutting a highly rich biodiversity area, poses significant risks to biodiversity, especially as it traverses environmentally sensitive areas, such as biological reserves, national parks, and Indigenous lands (Ferrante et al. 2021). The associated increase in traffic and deforestation makes it urgent to investigate the factors contributing to wildlife roadkill in the region. Thus, our study addresses a critical gap by analyzing the temporal variability of roadkill hotspots along BR-319 and how these relate to climatic and landscape variables across multiple spatial scales.

So, our study aimed to investigate temporal changes in roadkill hotspots over four years along the BR-319 highway and identify the climatic factors (temperature and precipitation) and landscape characteristics (land cover measured at different scales) influencing roadkill events. We hypothesized that: (1) the dynamics of hotspots will vary annually, and (2) higher temperatures would increase roadkill involving mammals and reptiles, while (3) rainy periods would heighten collisions involving amphibians, as flooded areas would increase roadkill involving within this group. For landscape, (4) agricultural areas would lead to higher roadkill numbers for birds and mammals, while (5) forest cover would positively influence roadkills for all taxa due to the species movement between both sides of the highway.

Methodology

Study area

The road BR-319 is a federal highway in Brazil that connects the states of Amazonas (AM) and Rondônia (RO), serving as a strategic link between the northern region and the rest of the country. Spanning approximately 877 km, the highway starts in Manaus (AM) and ends in Porto Velho (RO), crossing regions of high environmental and socioeconomic importance in the Amazon. Along its route, it intersects major rivers like the Madeira and Purus, which are critical for local biodiversity and fluvial transport. Additionally, the highway passes near protected areas such as the Rio Trombetas Biological Reserve and the Mapinguari National Park. Despite its strategic role, BR-319 faces challenges related to environmental conservation and road maintenance, particularly during the rainy season when unpaved sections become impassable (Fearnside et al. 2006).

Constructed in the 1970s as part of a national integration plan, BR-319 connects the "arc of deforestation" in the southern Amazon to Manaus, a central hub of the region (Fearnside et al. 2006). After partial abandonment in 1988, much of the highway became impassable, especially the "Middle Section" (km 250 to 655.7), characterized by a lack of paving and deteriorated conditions. In 2015, a maintenance program temporarily restored access to some parts during the dry season. Today, the highway is divided into four segments: the deteriorated "Middle Section", the Segment A (km 0 to 177.8) and Segment C (km 177.8 to 250), which are partially paved, and Segment B (km 655.7 to 877.4), which is fully paved. Our study focused on Segment A of BR-319, covering the first 177.8 km from Manaus to the municipalities of Careiro da Várzea and Manaquiri, in Amazonas (Fig. 1). Traffic data is not available for

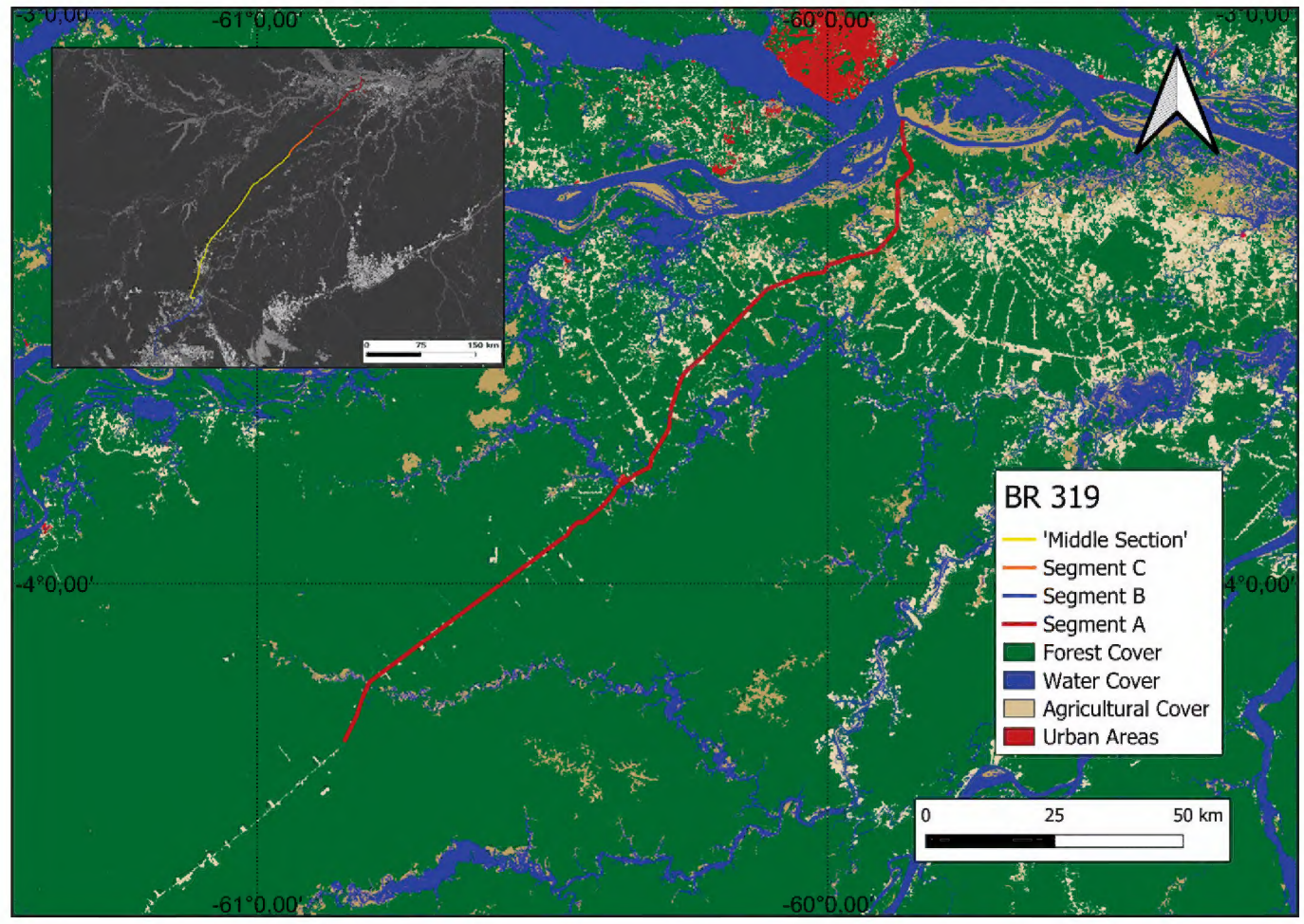

Figure 1. Segment A of BR-319 (in red), representing the current study's area of interest. At the upper left corner, there is a general view of BR-319 in its entirety, starting in Manaus (north) and ending in Porto Velho (south), with each segment highlighted in distinct colors.

BR-319, but segments A and B experience higher traffic volumes than segment C. This is likely because segments A and B are located near major urban centers—Manaus in segment A, the largest city in the Amazon, and Porto Velho in segment B. BR-319 is the only highway connecting Manaus to southern and southeastern Brazil, concentrating the flow of trucks coming from those regions. However, during periods of heavy rainfall, traffic tends to decrease or even come to a complete halt, especially for trucks. This is particularly true for segment C, which is unpaved and often becomes impassable during the rainy season due to poor road conditions, severely disrupting truck traffic.

Fauna roadkill database

Our study utilized a roadkill database from BR-319, provided by the Brazilian National Department of Transport Infrastructure (DNIT). The database was developed based on monthly data collection conducted from October 2017 to September 2021, covering 48 months of sampling and spanning four annual seasonal cycles. Throughout the study period, there were minimal changes in the data collection team. Whenever team members were replaced, standardized training was conducted to ensure methodological consistency and reduce potential observer bias. This approach helped maintain data reliability across all years of the study. The records include individuals identified, when possible, to the species level, and were grouped into four focal taxonomic groups for analysis: amphibians, reptiles, birds, and mammals. The database was compiled through roadkill monitoring using a vehicle traveling at an average speed of 40 km/h. Total monitoring time per survey varied due to the time required for carcass collection and data recording. Monitoring was carried out along the highway on one-way trips, with stops made to collect additional data whenever carcasses or live animals were observed. All recorded carcasses were photographed, and those

found on the road, or its shoulders were classified as roadkill events. Identifications were performed, when possible, by specialist biologists in herpetology, ornithology, and mammalogy. Each carcass was georeferenced to obtain precise coordinates. Other data collected during the survey, such as carcass persistence and live fauna observations, were not included in this study, as they fell outside the main research scope. Additionally, logistical constraints prevented the consistent implementation of carcass removal experiments, which limited the ability to incorporate these data into the present analysis.

For this study, we selected only georeferenced records along Segment A of BR-319, specifically between kilometers 0 and 177.8 of the highway, focusing on the northern paved section. We included only records where identification was possible to at least the class level, which allowed classification within the analyzed taxonomic groups. Records of domestic or exotic species (e.g., *Canis familiaris*, *Equus caballus*, *Felis catus* and *Gallus domesticus*) were excluded to focus on the impacts of the studied factors on native fauna.

Climatic and landscape variables

We collected monthly accumulated precipitation and mean monthly temperature data from the Brazilian National Meteorological Institute (INMET 2023) for station 82331 in Manaus, AM. This station was selected due to its relative proximity to the monitored section of BR-319, particularly segments A and part of B, and because it provided the most complete and continuous data series for the entire study period (October 2017 to October 2021). Other available stations were located at significantly greater distances—often over 200 km from the study area—or lacked consistent long-term data. While we acknowledge that localized climate conditions along the highway may vary due to differences in vegetation cover, topography, and water bodies, the use of station 82331 offers a consistent and reliable regional proxy for broader climatic trends. As such, we calculated the mean monthly temperature for each month between October 2017 and October 2021 based on daily mean temperatures.

For landscape variables, we assessed the coverage of flooded areas, forest cover, and agricultural areas surrounding the highway. We obtained the data using georeferencing techniques in QGIS (QGIS Development Team 2024) and surface maps with 15-meter resolution provided by the MapBiomas project (MapBiomas Project 2023). We divided the Segment A of the highway, spanning 177.8 km, into 1-km sections, and buffers of 500 m, 1000 m, 5000 m, and 10000 m were created around each section. We choose these buffer sizes based on their recurrent use in previous studies focused on wildlife-vehicle collisions and landscape effects (Bueno et al. 2015; Bencin et al. 2019; Filius et al. 2020; Hamer et al. 2021; Pinto et al. 2023).

The use of multiple buffer sizes allows us to explore scale-dependent effects of land cover on roadkill patterns and to capture the ecological variation among different taxonomic groups. Smaller buffers (500 m and 1 km) are expected to better reflect immediate habitat conditions affecting less mobile species, such as amphibians and small reptiles, which typically move shorter distances and respond to fine-scale environmental features (Bueno et al. 2015; Filius et al. 2020). In contrast, larger buffers (5 km and 10 km) account for broader habitat use by more mobile taxa, such as birds and large mammals, whose movements

and home ranges may extend several kilometers from the road (Freitas et al. 2013; Medrano-Vizcaíno et al. 2022). This multiscale approach helps us identify the most ecologically relevant spatial scale at which landscape composition influences roadkill occurrences for each group.

Using these buffers, we calculated the percentage of coverage for each land cover type. This was done through vector area calculations in QGIS, with distinct color bands for each surface type. We identified these values for each year of the study to ensure that roadkill records were associated with temporally relevant and representative local landscape conditions.

Statistical analyses

To analyze hotspots, we divided the 48 months of sampling into four 12-month periods, starting in October of the first year and ending in September of the following year, repeating the process for subsequent years. We performed analyses for all roadkill data combined and separately for each fauna group (birds, mammals, reptiles, and amphibians). We chose to analyze annual cycles of roadkill because this timeframe is typically used in both scientific literature (e.g., Santos et al. 2022; Mota et al. 2023) and to define roadkill hotspots for implementing mitigation measures in Brazil.

To identify roadkill hotspots, we applied the 2D Ripley K-statistics test using Siriema 2.0 software, which evaluates aggregation intensity at different spatial scales through the L(r) function. L(r) values exceeding the upper confidence limit (UCL) indicate significant clustering, while values below the lower confidence limit (LCL) suggest significant dispersion. The test was configured with an initial radius of 500 meters, 500-meter increments, a 95% confidence level, and 999 simulations (Mota et al. 2023). These parameters were selected to correspond to relevant spatial scales for implementing effective mitigation measures along the studied segment and the entire BR-319 (Mota et al. 2023).

To identify segments with high concentrations of roadkill (hotspots), we used the 2D hotspot test in Siriema 2.0. This test evaluates the spatial distribution of events along the highway, using the Hotspot Score (HS) as a measure of aggregation intensity. Significant hotspots are identified by HS values above the UCL, while values within the confidence limits suggest random distribution. The test was performed annually for each fauna group to assess temporal variation in hotspots. The radius was set to 500 meters, with 1000 simulations and a 95% confidence interval.

To investigate the influence of climatic and landscape factors on roadkill, we performed generalized linear models (GLMs), initially adjusted for a Poisson distribution. We tested for over-dispersion in all models and, when detected, used quasi-Poisson or negative binomial models accordingly. In cases of poor fit or non-independence of observations, particularly across years or road sections, we also tested generalized linear mixed models (GLMMs) with random effects for year and/or road segment to account for temporal and spatial non-independence. Analyses were conducted in R software (R Core Team 2024).

We built separate models for climatic and landscape factors, with the number of roadkill events as the response variable (considering each fauna group separately). We tested all predictor variables for multicollinearity. For climatic models, we used mean monthly precipitation, mean monthly temperature, and their interaction

as predictors' variables. For landscape models, we included the proportions of water, forest, and agricultural cover, along with their interactions, as predictors' variables. We constructed models for each buffer (500 m, 1000 m, 5000 m, and 10000 m) to identify specific landscape influences on roadkill at different spatial scales. To assess model assumptions, we tested the normality of residuals using the Shapiro-Wilk test and homogeneity of variances using Levene's test. For final model performance, we calculated a deviance-based pseudo-R^2 for each generalized linear model (GLM). For quasi-Poisson models, pseudo-R^2 was manually calculated as 1 − (model deviance/null deviance), providing a measure of explained variance.

Results

Our data comprised a total of 673 roadkill incidents. In the first year, 56 reptiles, 27 mammals, 42 birds and 51 amphibian individuals were registered. In the second, 68 reptiles, 30 mammals, 46 birds and 44 amphibians were registered. In the third, 78 reptiles, 23 mammals, 36 birds and 46 amphibians were registered. Finally, in the fourth year, 49 reptiles, 27 mammals, 28 birds and 22 amphibians were registered (Table 1). During the 48-month data collection period in Segment A of BR-319, 107 mammals, 251 reptiles, 152 birds, and 163 amphibians were recorded as roadkill. The species with the highest number of roadkill events registered were *Rhinella marina* (Cane toad), *Didelphis marsupialis* (Common opossum) and *Coragyps atratus* (Black vulture) with 133, 85, and 44 individuals, respectively. The highest average temperature recorded was 31.2 °C in October 2018, while the lowest was 27.3 °C in March 2021, with a monthly average of 28.8 °C. Rainfall ranged from 19.5 mm in August 2018 to 589.3 mm in March 2021, with an overall average of 226.5 mm during the study period. Landscape analysis revealed a wide variety of vegetation cover types. Forest cover ranged from 38.4% to 98.9%, with the lowest percentages observed in the 5000 m and 10000 m buffers. Agricultural cover varied from 0% to 75%, showing the greatest variation in the 500 m and 1000 m buffers. Wetland coverage ranged from 0% to 37.7%, with minimal variation across the buffers (see Suppl. material 1).

Roadkill hotspots

For all wildlife groups combined, we observed a significant concentration of hotspots within the first 30 kilometers of the highway, which was the most consistently affected area throughout the analyzed periods. In year 1 (2017/2018), hotspots were located at kilometers 4, 7–9, 11–12, 14–15, 17, 19–21, 48, 58–59, and 81. In year 2 (2018/2019), hotspots occurred at kilometers 4, 6, 10, 12–16, 19, 43, 55–56, 59, 68, 80, 82–83, and 105. In year 3 (2019/2020), hotspots were identified at kilometers 3–4, 6–7, 9, 11–15, 25–26, 28–29, 34–35, and 44. In year 4 (2020/2021),

Table 1. Total number of roadkills registered for each group during each year of the study.

Group	Roadkills – Year 1	Roadkills – Year 2	Roadkills – Year 3	Roadkills – Year 4
Amphibians	51	44	46	22
Reptiles	56	68	78	49
Birds	42	46	36	28
Mammals	27	30	23	27

hotspots were found at kilometers 0, 2, 4, 6, 8–9, 19, 28, 30, 32–33, 48, 55, 63, and 71 (Fig. 2). Over the years, the most consistent hotspots were at kilometers 4, 6, 19, and 48, which stood out for their recurrence across all monitoring periods.

For amphibians, hotspots were located at kilometers 9, 12, 19–20, 58–59, and 71 in year 1. In year 2, hotspots were recorded at kilometers 13, 17, 19, 41, 55–56, 59, 66, and 68. In year 3, hotspots occurred at kilometers 7, 9, 11, 18, 28–29, 35, 37, 44, 48, and 69. In year 4, a single hotspot was identified at kilometer 1 (Fig. 4). The most consistent hotspots over the years were at kilometers 9, 19, and 59, which appeared in at least two of the four periods analyzed.

For reptiles, annual variation in hotspot location patterns was relatively low, with over 95% of hotspots concentrated between kilometers 3 and 30. In year 1, hotspots were identified at kilometers 5, 8, 11, 14–17, 38, 51, and 83. In year 2, hotspots were recorded at kilometers 4–5, 10, 13–16, and 22. In year 3, hotspots occurred at kilometers 2–3, 6–7, 9, 12–15, 19–20, 26, 29, and 44. In year 4, hotspots were found at kilometers 2, 4, 6–9, 12–14, 28, 33, and 55 (Fig. 5). The most consistent hotspots over the years were at kilometers 2, 4, 6, 12, and 14, which appeared in at least three of the four periods analyzed.

For birds, we observed a significant variation in hotspot distribution patterns across annual monitoring cycles. In year 1, hotspots were located at kilometers 19, 48, 80, and 84. In year 2, hotspots occurred at kilometers 6, 14, 43, 55, 68, 70, and 82. In year 3, hotspots were identified at kilometers 25, 34, 36, and 44. In year 4, hotspots were at kilometers 1, 19, 30, 48, and 75 (Fig. 3). The most consistent hotspots over the years were at kilometers 19 and 48, appearing in three of the four periods analyzed.

Mammals exhibited the most distinct aggregation pattern among the taxonomic groups studied. While variation in hotspot locations was relatively

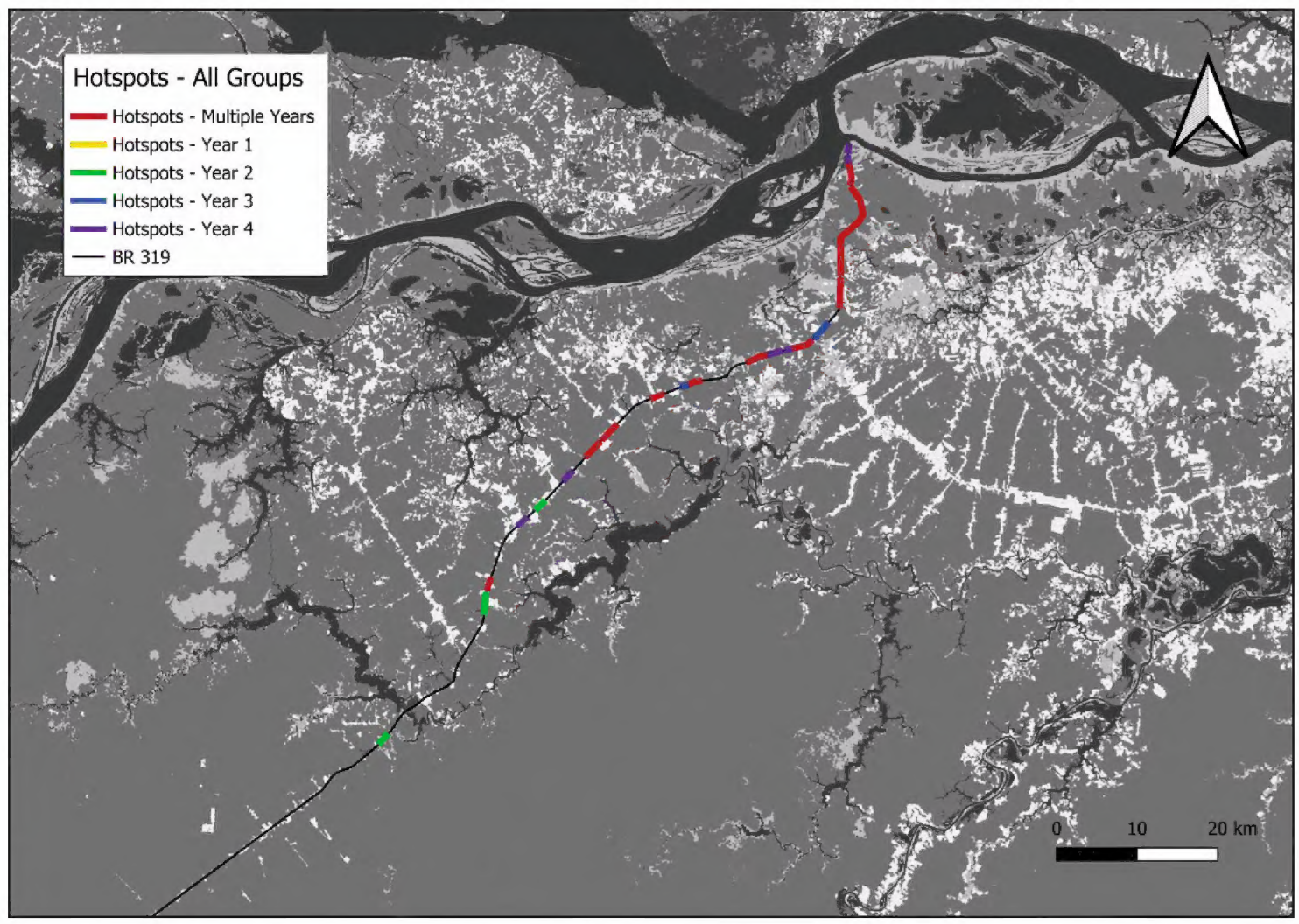

Figure 2. Hotspots (including all faunal groups) along 'Segment A' of BR 319 in each year between 2017 and 2021 and multiple years. Hotspots which repeat along our study are colored in red and represent all areas which share a hotspot location for two or more years. All other hotspots are highlighted in distinct colors indicating the respective years in which they were found.

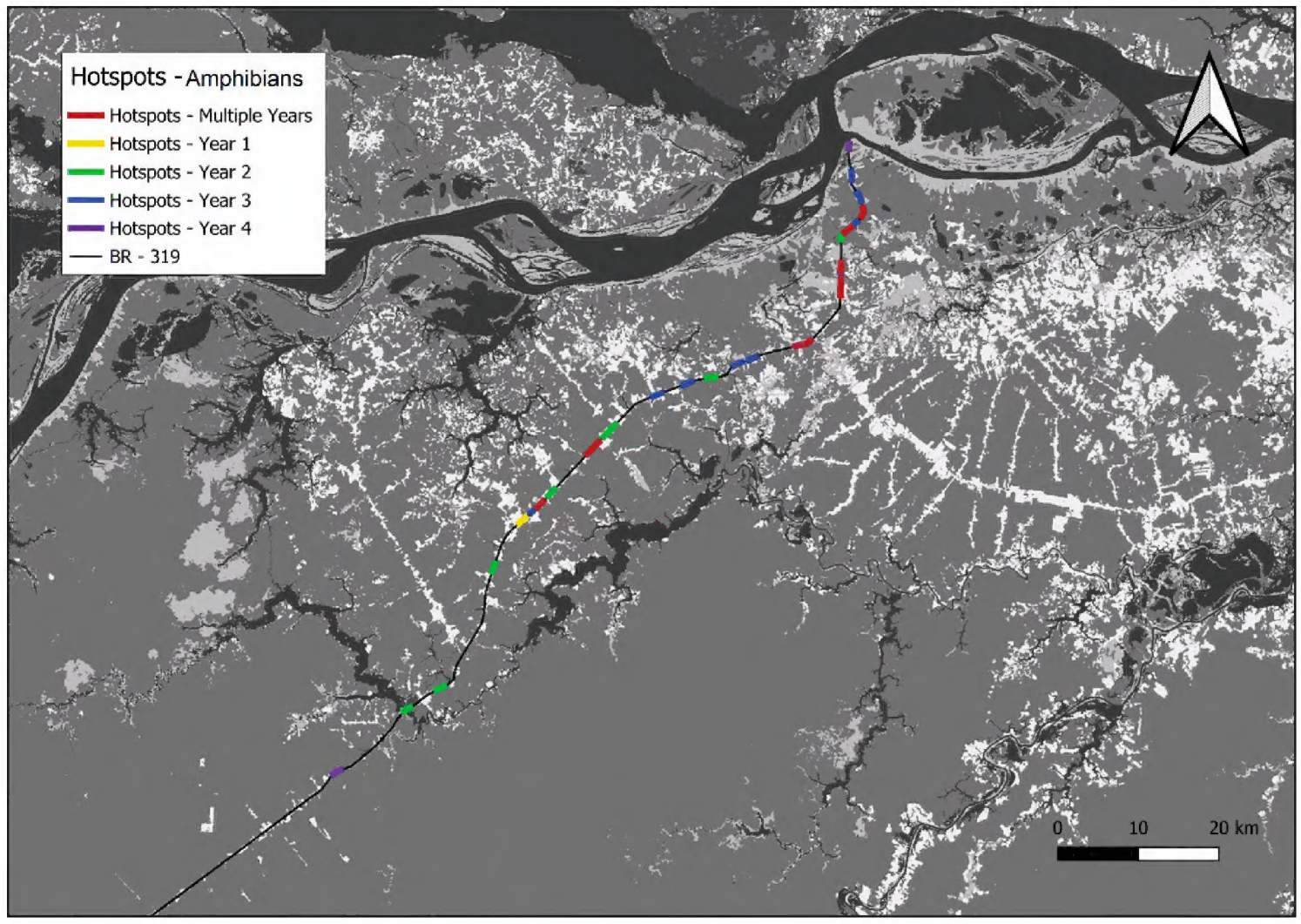

Figure 3. Hotspots of amphibians along 'Segment A' of BR 319 in each year between 2017 and 2021 and multiple years. Hotspots which repeat along our study are colored in red and represent all areas which share a hotspot location for two or more years. All other hotspots are highlighted in distinct colors indicating the respective years in which they were found.

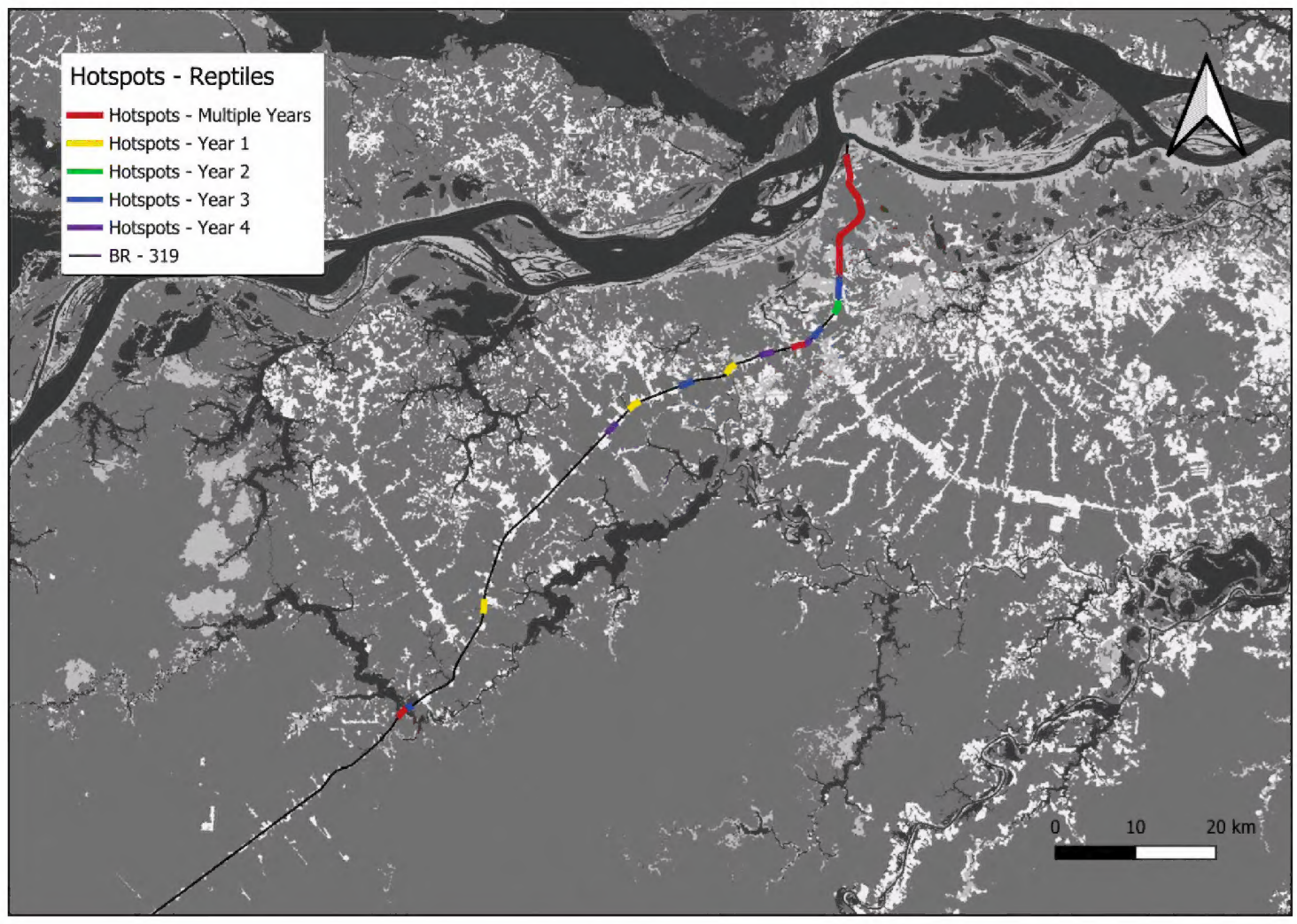

Figure 4. Hotspots of reptiles along 'Segment A' of BR 319 in each year between 2017 and 2021and multiple years. Hotspots which repeat along our study are colored in red and represent all areas which share a hotspot location for two or more years. All other hotspots are highlighted in distinct colors indicating the respective years in which they were found.

small over the analyzed periods, mammal hotspots were less frequent compared to other groups. In year 1, hotspots were recorded at kilometers 76, 80, and 94. In year 2, they were at kilometers 63, 80, and 107. In year 3, hotspots

were located at kilometers 11, 28, and 99. In year 4, hotspots occurred at kilometers 9 and 32 (Fig. 6). The most consistent hotspots were at kilometers 80 and 107, appearing in two consecutive periods.

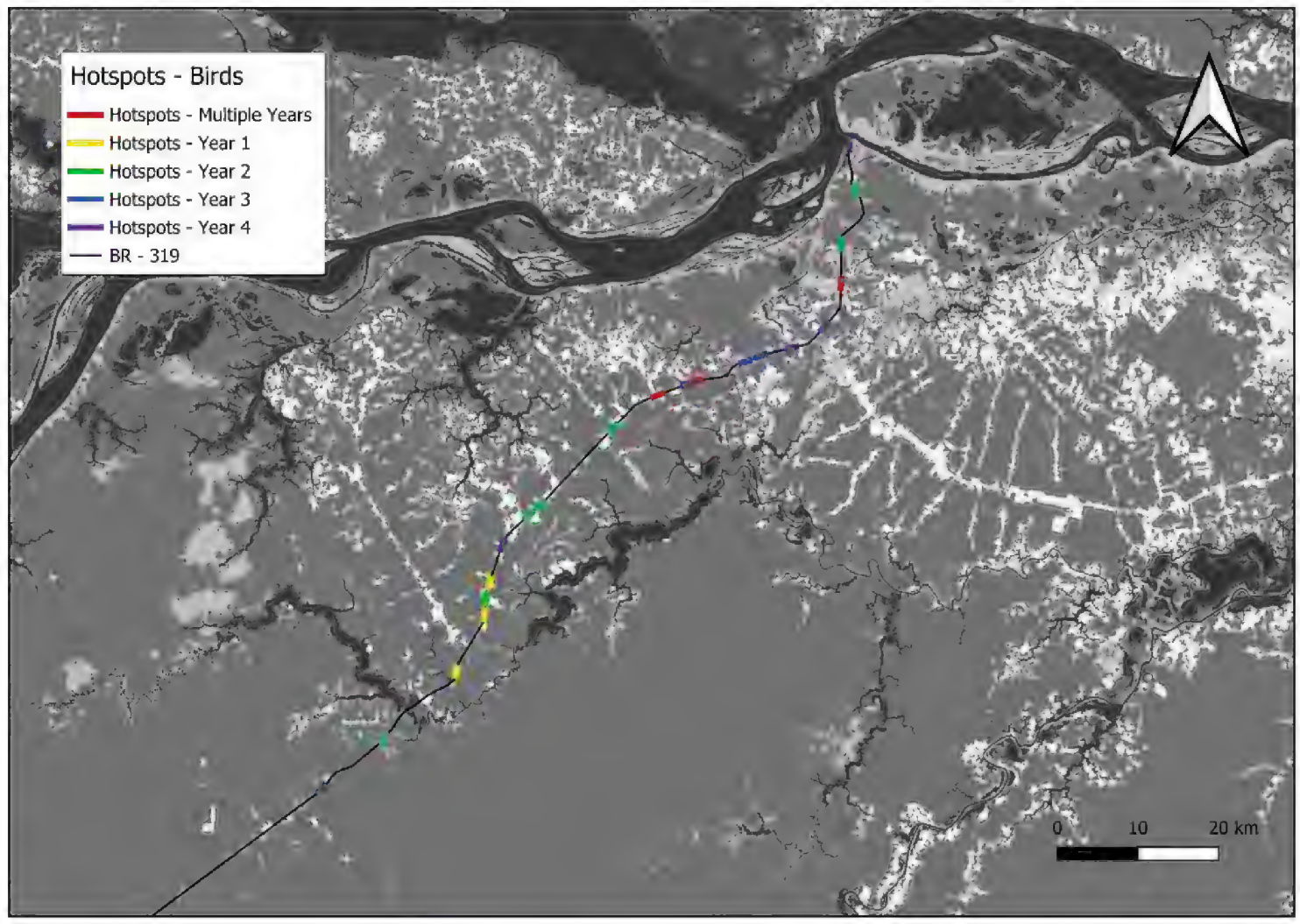

Figure 5. Hotspots of birds along 'Segment A' of BR 319 in each year between 2017 and 2021 and multiple years. Hotspots which repeat along our study are colored in red and represent all areas which share a hotspot location for two or more years. All other hotspots are highlighted in distinct colors indicating the respective years in which they were found.

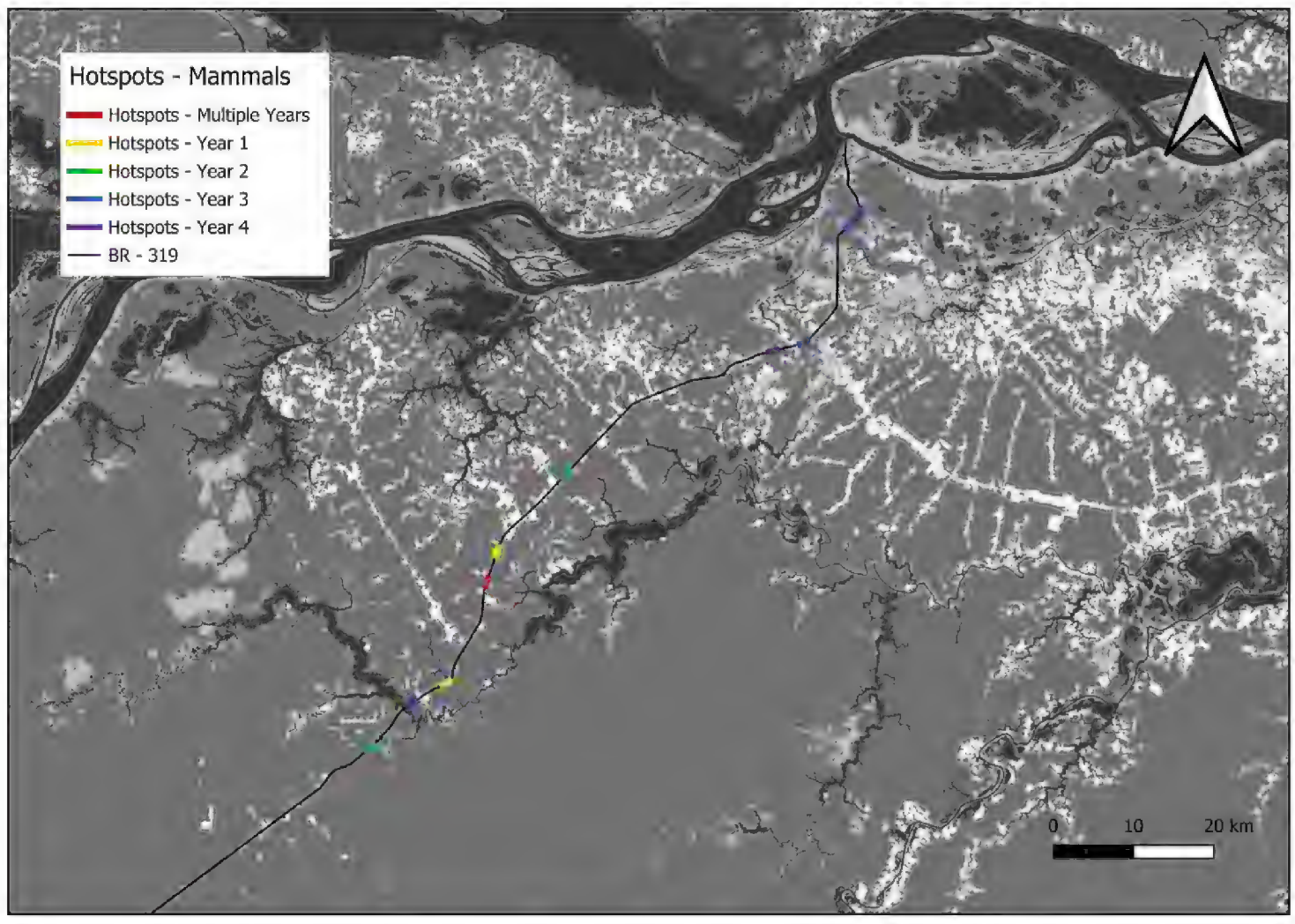

Figure 6. Hotspots of mammals along 'Segment A' of BR 319 in each year between 2017 and 2021 and multiple years. Hotspots which repeat along our study are colored in red and represent all areas which share a hotspot location for two or more years. All other hotspots are highlighted in distinct colors indicating the respective years in which they were found.

Table 2. Final models of climatic variables on the different groups analyzed. Variables of Rainfall and Temperature are in millimeters and Celsius respectively. Beta values determine the direction and intensity of the interaction found, and VIF values determine the variable's correlation coefficient.

Group	Final Model	Variables	Beta	P-value	VIF
Amphibians	Rainfall(mm) + Temperature(C)	Rainfall(mm)	-0.001511	3.30e-05	1.612
		Temperature(C)	0.738469	<2e-16	1.612
Reptiles	Rainfall(mm) + Temperature(C)	Rainfall(mm)	-0.001309	0.0237	1.591
		Temperature(C)	0.414551	1.10e-07	1.591
Birds	Rainfall(mm) + Temperature(C)	Rainfall(mm)	-0.001802	0.0165	1.553
		Temperature(C)	0.132322	0.2179	1.553
Mammals	Rainfall(mm) + Temperature(C)	Rainfall(mm)	-0.000016	0.9856	1.578
		Temperature(C)	0.3026	0.0197	1.578

Climate factors

Models assessing the influence of climatic conditions on roadkill revealed similar patterns across the groups. Both temperature and precipitation were found to influence roadkill, with all groups showing a positive correlation between higher temperatures and an increase in roadkill incidents. In contrast, a negative correlation between rainfall and roadkill was observed, indicating that higher rainfall decreased roadkill across all groups (Table 2).

Landscape factors

Our adjusted models evaluating the impact of landscape factors on roadkill numbers revealed some common patterns across all studied groups. In buffers of 500 m, 1000 m, and 5000 m, all groups showed a negative relationship between water and forest cover, meaning that an increase in the proportion of these landscape types consistently led to a reduction in roadkill numbers across all groups within these buffers (Table 3). The pseudo-R^2 values for the final GLMs ranged from 0.05 to 0.38. Birds and mammals exhibited lower explanatory values (pseudo-R^2 between 0.05 and 0.11), while amphibians showed moderate values (pseudo-R^2 between 0.09 and 0.17), and reptiles demonstrated higher explanatory power (pseudo-R^2 between 0.26 and 0.38).

Amphibians and reptiles exhibited lower roadkill in segments with higher water, forest, and agricultural cover in buffers ranging from 500 m to 5,000 m for amphibians and from 500 m to 10,000 m for reptiles. However, the interaction between forest and water covers increased amphibian roadkill in the 500 m buffer. At the 5,000 m buffer, interactions between forest and agricultural cover led to an increase in amphibian roadkills, while at the 10,000 m buffer, interactions between water and agricultural cover were associated with higher roadkill rates. For reptiles, the interaction between water and either forest or agricultural cover, depending on the buffer scale, was linked to increased roadkill (Table 3).

For bird roadkill models, an increase in water, forest, and agricultural cover reduced roadkills in all buffers below 10,000 m. However, the interaction between forest and agricultural cover had a positive effect on bird roadkills in the 1,000 m and 5,000 m buffers. At the 10,000 m buffer, roadkill rates were higher in areas with greater water and agricultural cover. Mammal roadkill decreased

Table 3. Final models of landscape factors. Beta values determine the direction and intensity of the interaction found, and VIF values determine the variable's correlation coefficient.

Buffer	Final Model	Variable	Beta	P-value	VIF
AMPHIBIANS					
500 m	Water% * Forest% + Water% + Forest% + Farming%	Water%	-21.7463	5.33E-05	6.95172
		Farming%	-1.6147	0.001895	1.917455
		Forest%	-3.9207	2.88E-12	2.345862
		Water%: Forest%	51.5117	0.000114	6.836359
1000 m	Water% + Forest% + Farming%	Water%	-7.5614	0.0013	1.38471
		Farming%	-1.7397	0.01161	1.9823
		Forest%	-3.7708	1.05E-12	1.902177
5000 m	Water% + Forest% + Farming% + Forest%:Farming%	Water%	-8.447	0.000379	3.976797
		Farming%	-15.729	0.001005	28.408446
		Forest%	-7.603	1.68E-08	6.731983
		Forest%: Farming%	20.832	0.001125	16.679164
10000 m	Forest% + Water%:Farming% + Forest%:Farming%	Forest%	-1.6125	0.02514	2.270518
		Water%: Farming%	82.611	7.42E-05	2.186837
		Forest%: Farming%	5.0727	0.00517	1.057216
REPTILES					
500 m	Water% * Forest% + Forest% * Farming%	Water%	-20.6889	3.83E-07	5.507039
		Farming%	-3.9067	2.12E-05	5.890916
		Forest%	-3.7996	2.60E-15	3.158706
		Water%: Forest%	22.3236	0.03157	5.12831
		Forest%: Farming%	-6.8554	0.00496	4.455429
1000 m	Water% * Farming% + Forest%	Water%	-19.2198	2.13E-13	2.286869
		Farming%	-8.951	<2e-16	2.04495
		Forest%	-6.1661	<2e-16	1.463817
		Water%: Farming%	79.7469	0.00277	2.225765
5000 m	Water% * Forest% + Farming%	Water%	-22.7703	<2e-16	11.843587
		Farming%	-12.3421	<2e-16	3.298776
		Forest%	-12.0117	<2e-16	3.292301
		Water%: Forest%	23.6916	8.05E-06	9.173043
10000 m	Water% * Farming% + Forest%	Water%	-6.012	0.021387	14.067148
		Farming%	-8.305	0.000531	7.263759
		Forest%	-6.843	9.70E-07	13.61752
		Water%: Farming%	96.25	2.71E-10	2.058495
BIRDS					
500 m	Water% + Farming% + Forest%	Water%	-4.585	0.046	1.39641
		Farming%	-0.9395	0.0791	1.943157
		Forest%	-3.1872	3.78E-10	1.799457
1000 m	Water% + Farming% + Forest% + Farming%:Forest%	Water%	-6.3727	0.01121	1.567648
		Farming%	-5.026	0.01348	11.861648
		Forest%	-4.9968	3.31E-08	3.509808
		Farming%: Forest%	9.0213	0.01963	8.015893
5000 m	Water% + Farming% + Forest% + Farming%:Forest%	Water%	-5.597	0.040945	4.52805
		Farming%	-11.346	0.028166	29.97342
		Forest%	-5.585	0.000267	7.78822
		Farming: Forest%	16.873	0.011852	16.53537
10000 m	Water% + Farming%	Water%	4.573	1.37E-05	1.114696
		Farming%	6.1599	2.81E-10	1.114696

Buffer	Final Model	Variable	Beta	P-value	VIF
MAMMALS					
500 m	Water% * Forest%	Water%	-14.6222	0.009368	6.8865
		Forest%	-1.9201	0.000646	1.493346
		Water%: Forest%	53.8061	0.000242	6.874451
1000 m	Water% * Forest% + Forest% * Farming%	Water%	-30.585	0.00591	16.158736
		Farming%	-11.417	0.001407	19.879832
		Forest%	-5.139	0.000211	4.464162
		Water%: Forest%	65.265	0.000772	14.637192
		Forest%: Farming%	21.157	0.000431	13.904734
5000 m	Water% * Forest% + Forest% * Farming%	Water%	-21.573	0.00601	18.711745
		Farming%	-23.806	0.003355	44.039104
		Forest%	-5.397	0.008825	7.920169
		Water%: Forest%	35.625	0.004276	15.541902
		Forest%: Farming%	34.531	0.000484	27.239535
10000 m	Water% * Forest%	Water%	-27.9559	1.78E-06	11.61376
		Forest%	-3.1046	0.000639	2.022671
		Water%: Forest%	61.5738	1.52E-08	9.000986

with higher water and forest cover in all analyzed buffers. However, roadkill also decreased with higher agricultural cover in the lowest and highest buffers analyzed (500 m and 10,000 m). The interaction between agriculture and forest cover increased mammal roadkill at the 1,000 m and 5,000 m buffers, while the interaction between forest and water cover increased roadkill at the 5,000 m and 10,000 m buffers (Table 3).

Discussion

The temporal variation of roadkill hotspots along BR-319 confirms the complexity of factors influencing wildlife mortality on highways. Our results largely support the initial hypothesis that roadkill hotspots would vary annually, with high roadkill areas not remaining constant over time. However, certain locations, such as kilometers 4, 6, 19, and 48, emerged as recurring hotspots across multiple groups. Those hotspots are in significantly fragmented areas, which border, and partially include the outer region of the city of Manaus. Even in smaller spatial scales, the environment around those kilometers shows a strong urban and agricultural presence, which indicates they may also be in particularly heavy traffic areas. Three of those points are also located before and relatively nearby to the Araçá river bridge, a road feature which could serve as a wildlife attractive and influence roadkill patterns alongside nearby areas (Fraga et al. 2022).

The variation in most of the hotspot locations across taxonomic groups could be related to the ecology of the species. While mammals and birds have more dispersed hotspots that vary over the years, amphibians and reptiles show more consistency in their hotspot locations. This is likely because amphibians and reptiles move less across the landscape compared to birds and mammals and tend to be more faithful to their breeding areas (Taylor et al. 2020; Fischer 2023). The consistency of hotspots in certain sections aligns with the behavior of these species, which tend to concentrate in specific areas due to microclimatic conditions (Inman et al. 2022; Fischer 2023), where they are more vulnerable to roadkill (Gomes et al. 2023; Costa and Gomides 2024). In contrast, mammals, with their broader movement patterns, exhibit roadkill fluctuations as they cover larg-

er areas and have overlapping territories across different segments of the road (Teitelbaum and Mueller 2019). This explains why mammal hotspots are more dispersed and less consistent over the years compared to other groups. Even indirectly related events, such as changes in road traffic during the COVID-19 pandemic (Naveca et al. 2021), can impact highly responsive species. The pandemic caused frequent reductions in traffic, particularly in heavily affected areas like Amazonas (Naveca et al. 2021). Studies show these reductions had a varied effect on wildlife-vehicle collisions, sometimes increasing roadkills, but in other cases, having little or even a negative impact (Pokorny et al. 2022).

The hotspots are shaped by a dynamic interplay of climatic factors, landscape characteristics and animal behavior (Taylor et al. 2020) and we show that hotspot distribution in BR-319 is influenced by both microclimatic and landscape conditions, which shape roadkill patterns. These findings align with studies highlighting the influence of these factors on roadkill patterns (Grilo et al. 2009; Bueno and Almeida 2019; Medinas et al. 2021; Batista et al. 2022). Amphibians and reptiles exhibited a negative correlation with rainfall, contrary to our initial hypothesis regarding amphibians. One possible explanation is the increased frequency of adverse weather events along BR-319, particularly during the rainy season, which reduces traffic flow (Li et al. 2022). The road's limited infrastructure and occasional flooding may exacerbate these effects by deteriorating road conditions and slowing down vehicles, further influencing wildlife mortality. Even though the section we studied is already paved, the lack of paving in other sections may inhibit the overall use of the road. Additionally, heavy rainfall may lead to carcass washout or accelerated decomposition, especially among smaller species (Ratton et al. 2014). Both amphibians and reptiles also showed higher roadkill numbers with increased temperatures, aligning with previous studies on reptiles, particularly those exhibiting thermoregulatory behavior (Laurance et al. 2009; Grilo et al. 2011). However, this pattern was less evident in amphibians, likely due to the unique seasonal dynamics of the Amazon. The Amazonian summer (June to November) is characterized by a pronounced dry season, followed by intense rainfall during the Amazonian winter (December to May), which may counteract the effects of persistently high temperatures. In this region, the interaction between temperature and rainfall is complex. During the dry season, high temperatures coincide with much lower rainfall, creating a distinct microclimate with reduced soil moisture and humidity (Löbs et al. 2020). This differs from subtropical and temperate regions, where increased temperatures alone drive more predictable seasonal roadkill patterns, particularly when combined with summer rainfall. In the BR-319 region, amphibians may be less susceptible to higher roadkill rates during hot months because their activity is influenced by additional factors, such as lower humidity or reduced availability of suitable breeding sites in the landscape (Taylor et al. 2020).

For mammals and birds, climatic influences are often more species-specific, which has led to studies where aggregating species within broader groups fails to identify environmental causes for increased roadkill or only reveals correlations between roadkill patterns and species-specific physical or behavioral traits (Rowe et al. 2015; Kent et al. 2021; Medrano-Vizcaíno et al. 2022). However, some studies have also linked roadkill in both groups to higher rainfall and temperatures, suggesting that the composition of these groups can significantly impact their interactions with the local environment (Carvalho et al. 2015).

Our results also reveal that forest cover consistently reduces roadkill across all species groups and spatial scales. This suggests that forested areas act as natural barriers, potentially limiting wildlife movement near roads and thereby decreasing the likelihood of roadkill. Interestingly, this contrasts with findings from more fragmented regions, where greater forest cover has been associated with increased roadkill due to enhanced habitat connectivity and the need for animals to crossroads more frequently(Filius et al. 2020; Batista et al. 2022; Medrano-Vizcaíno et al. 2022). In such landscapes, animals are often forced to move between isolated patches in search of food, mates, or shelter, increasing their exposure to roads (Almeida-Corrêa et al. 2020; Fraga et al. 2022). Conversely, in well-preserved environments like those found along BR-319, the availability of contiguous habitat may reduce the need for long-distance movements, thereby lowering roadkill risk. Similarly, wetlands and agricultural areas were generally associated with lower roadkill rates across most species groups. This may indicate a broader effect of less urbanized landscapes creating a type of 'buffer zone' that discourages frequent road crossings.

Amphibians and reptiles, which are particularly sensitive to microclimatic shifts, experienced higher roadkill rates near areas where wetlands and agricultural land intersect, especially in the more urbanized zones along the first third of the road. This trend, evident only within the 500 m buffer scale, aligns with findings from previous studies, which identified roadkill patterns in these groups predominantly within smaller spatial scales, especially in amphibians (Laurance et al. 2014; Kouris et al. 2024). This can be attributed to their limited locomotion, reliance on specific microclimates, and preference for smaller landscape structures (Taylor et al. 2020; Medrano-Vizcaíno et al. 2022; Gonçalves et al. 2023; Kouris et al. 2024). Supporting this observation, our results show that the influence of wetlands and agricultural areas on roadkill rates in amphibians diminishes at larger spatial scales, vanishing entirely at the largest scale of 10,000 m.

It is also important to acknowledge that other variables not directly assessed in this study—such as habitat connectivity, availability of food resources, road topography, and both traffic volume and vehicle speed—can significantly influence roadkill patterns (Medrano-Vizcaíno et al. 2023), which could explain the low adjustment values of some of the GLM models in this study. For example, lower connectivity may compel animals to cross roads more frequently to access distant habitat patches (Batista et al. 2022), increasing mortality risk. Similarly, elevated traffic volumes and high vehicle speeds are commonly linked to increased roadkill rates, especially for fast-moving or low-visibility species (Marques et al. 2022). BR-319's unique combination of fluctuating traffic conditions, varying road conditions, and large stretches of continuous forest may moderate or amplify these effects in ways that warrant further investigation. Incorporating such variables in future studies would help to better explain roadkill dynamics and potentially refine mitigation strategies.

However, it is important to remember that effects of scale can vary across groups, species and populations, emphasizing that landscape influences on roadkill are region-specific and may depend on local ecological conditions (Freitas et al. 2013). For birds, for example, we have found the most distinct interaction at the 10,000 m scale, in which both wetlands and agricultural coverage were found associated with an increased roadkill count. The majority of birds' roadkills identified in this study were scavenger species of vultures

(*Coragyps atratus*), which could indicate attraction to areas where other species suffered larger roadkill rates, such as we observed for amphibians around the paired wetlands and agricultural areas (Medrano-Vizcaíno et al. 2022).

Our study offers valuable insights into the spatiotemporal patterns of wildlife roadkill along BR-319. We find that preserving forest cover is the most effective strategy for mitigating roadkill in highly conserved areas like the Amazon, though this is challenging given that roads are major drivers of habitat fragmentation. It further underscores the critical importance of the political debate surrounding the paving of the BR-319 highway. Researchers have consistently warned that this project should not proceed without the prior establishment of robust governance policies to prevent deforestation and illegal occupation in the region (Fearnside et al. 2020). Our results also underscore the need for targeted conservation measures, such as wildlife corridors and ecological barriers, particularly in areas where fragmented landscapes intersect with high levels of human activity. These strategies are crucial for mitigating roadkill in the Amazon, a region characterized by unique and delicate ecosystems. The findings also emphasize the importance of considering both climatic and landscape factors in conservation planning, as these variables interact in complex ways that can influence wildlife mortality. By integrating these considerations into road planning and conservation efforts, we can better safeguard biodiversity and reduce the negative impact of infrastructure on wildlife in the Amazon.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

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Author contributions

Conceptualization: CR, PNNM. Data curation: PNNM. Formal analysis: PNNM. Investigation: GAMS, JRNC, ALFS, EAS, ENS, FMF, RS, MVCJ, AAA. Methodology: PNNM, CR.

Project administration: CR. Software: PNNM. Supervision: MG, CR. Validation: MG, CR. Visualization: PNNM. Writing - original draft: PNNM. Writing - review and editing: ALFS, MG, ENS, CR, MVCJ, AAA, RS, JRNC, GAMS, PNNM, FMF, EAS.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: **table S1.** Table of highest, lowest and average values found for each landscape type (water cover, farming land and wooded forest) within each individual buffer used. **table S2.** Complete list of species, as well as number of individual roadkill register of each species found, used in this study. **figure S1**. Monthly values of the average temperature calculated for each month of our study period, from October 2017 to September 2021. **figure S2**. Monthly values of the total accumulated precipitation for each month of our study period, from October 2017 to September 2021. **figure S3.** Roadkill aggregation graphs for amphibians in accordance with the 500 m radius used in our analysis for the first year of study. **figure S4.** Roadkill aggregation graphs for amphibians in accordance with the 500 m radius used in our analysis for the second year of study. **figure S5.** Roadkill aggregation graphs for amphibians in accordance with the 500 m radius used in our analysis for the third year of study. **figure S6.** Roadkill aggregation graphs for amphibians in accordance with the 500 m radius used in our analysis for the fourth year of study. **figure S7.** Roadkill aggregation graphs for birds in accordance with the 500 m radius used in our analysis for the first year of study. **figure S8.** Roadkill aggregation graphs for birds in accordance with the 500 m radius used in our analysis for the second year of study. **figure S9.** Roadkill aggregation graphs for birds in accordance with the 500 m radius used in our analysis for the third year of study. **figure S10.** Roadkill aggregation graphs for birds in accordance with the 500 m radius used in our analysis for the third year of study. **figure S11**. Roadkill aggregation graphs for mammals in accordance with the 500 m radius used in our analysis for the first year of study. **figure S12.** Roadkill aggregation graphs for mammals in accordance with the 500 m radius used in our analysis for the second year of study. **figure S13.** Roadkill aggregation graphs for mammals in accordance with the 500 m radius used in our analysis for the third year of study. **figure S14.** Roadkill aggregation graphs for mammals in accordance with the 500 m radius used in our analysis for the fourth year of study. **figure S15.** Roadkill aggregation graphs for reptiles in accordance with the 500 m radius used in our analysis for the first year of study. **figure S16.** Roadkill aggregation graphs for reptiles in accordance with the 500 m radius used in our analysis for the second year of study. **figure S17.** Roadkill aggregation graphs for reptiles in accordance with the 500 m radius used in our analysis for the third year of study. **figure S18.** Roadkill aggregation graphs for reptiles in accordance with the 500 m radius used in our analysis for the fourth year of study. **figure S19.** Hotspot distribution of all roadkill events included in our study (all groups) for the first year of study. **figure S20.** Hotspot distribution of all roadkill events included in our study (all groups) for the second year of study. **figure S21.** Hotspot distribution of all roadkill events included in our study (all groups)

for the third year of study. **figure S22.** Hotspot distribution of all roadkill events included in our study (all groups) for the fourth year of study. **figure S23.** Hotspot distribution of all roadkill events included in our study for amphibians for the first year of study. **figure S24.** Hotspot distribution of all roadkill events included in our study for amphibians for the second year of study. **figure S25.** Hotspot distribution of all roadkill events included in our study for amphibians for the third year of study. **figure S26.** Hotspot distribution of all roadkill events included in our study for amphibians for the fourth year of study. **figure S27.** Hotspot distribution of all roadkill events included in our study for birds for the first year of study. **figure S28.** Hotspot distribution of all roadkill events included in our study for birds for the second year of study. **figure S29.** Hotspot distribution of all roadkill events included in our study for birds for the third year of study. **figure S30.** Hotspot distribution of all roadkill events included in our study for birds for the fourth year of study. **figure S31.** Hotspot distribution of all roadkill events included in our study for mammals for the first year of study. **figure S32.** Hotspot distribution of all roadkill events included in our study for mammals for the second year of study. **figure S33.** Hotspot distribution of all roadkill events included in our study for mammals for the third year of study. **figure S34.** Hotspot distribution of all roadkill events included in our study for mammals for the fourth year of study. **figure S35.** Hotspot distribution of all roadkill events included in our study for reptiles for the first year of study. **figure S36.** Hotspot distribution of all roadkill events included in our study for reptiles for the second year of study. **figure S37.** Hotspot distribution of all roadkill events included in our study for reptiles for the third year of study. **figure S38.** Hotspot distribution of all roadkill events included in our study for reptiles for the fourth year of study.

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